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WHAT IS THE ANTIGEN RESPONSIBLE FOR THE ANTI-BODIES IN DORSET-NILES SERUM?

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WHAT IS THE ANTIGEN RESPONSIBLE FOR THE ANTI-BODIES IN DORSET-NILES SERUM?

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The Dorset-Niles serum is a protective serum against hog cholera. Aside from our knowledge of its protective power, what proof have we that this serum contains anti-bodies? Our researches have shown the presence of agglutinins for B. cholerae suis and protective substances against virulent cultures of this organism. However, it is not considered that B. cholerae suis is the etiologic factor of prime importance in the production of hog cholera. Therefore we should look upon the filterable virus as the antigen possessing greatest importance and we should seek to ascertain the specific anti-bodies resulting from the interaction of the filtered virus and the pigs treated.

In attempting to study the phenomena resulting from the interaction of antigen and the body cells or fluids it is advisable to divest each factor of all complicating elements. We have previously studied the Dorset-Niles serum and its agglutinative action on B. cholerae suis. This serum was from animals that had been treated with large doses of hog cholera virus blood. This virus blood is supposed to contain frequently both B. cholerae suis and the filterable virus, probably two distinct antigens.

In the production of anti-bodies in the blood of a pig these two antigens may exert influences independently, symbiotically or antibiotically. We have attempted to obviate complications by using an antigen composed of B. cholerae suis only or the filterable virus only.

We first attempted to hyperimmunize a pig by the use of normal pig blood and bouillon cultures of B. cholerae suis. The culture used was "virus 136" and has already been described.* The treatment of the pig employed in this experiment was as follows:

Virus pig 139, Duroc-Jersey, wt. 125 lbs. Injected 5 c.c. virus Expt. 199 (supposed to contain both B. cholerae suis and the filterable virus) 12-7-'09, into the muscles of the ham. The pig remained well. Failure to develop hog cholera is attributed to natural immunity since another pig from the same lot injected at the same time with a similar dose of the same virus developed a typical case of hog cholera in 8 days. On 12-22-'09, there was injected into the axillary region, 150 c.c. of defibrinated blood from Expt. pig 237 mixed with 50 c.c. of a 20 hour bouillon culture of B. cholerae suis.

No visible or palpable lesions produced.

On 12-30-'09 there was injected into the opposite axillary region 465

* See Tech. Bul. No. 3 and No. 8, Mich. Agrl. Expt. Sta.

c.c defibrinated blood from Expt. pig 323 B mixed with 100 c.c. of a 16 hour bouillon culture of B. cholerae suis.

On 1-4-'10 there was injected 150 c.c. of defibrinated blood from Expt. pig 255 mixed with 150 c.c. of an 18 hour bouillon culture of B. cholerae suis.

Pig thin but vigorous and good appetite; walks with stilted gait. No abscesses visible or palpable.

On 1-12-'10 drew 289 c.c. blood from tail, defibrinated and preserved in .5% phenol.

On 1-13-'10 injected 150 c.c. defibrinated blood from Expt. 193 mixed with 150 c.c. of a 16 hour bouillon culture of B. cholerae suis.

On the afternoon of this day the pig died. The only lesions were local and consisted in an infiltration and necrosis of the muscular and connective tissue between the shoulder and thorax. The tissues were greenish in color, spongy and saturated with a foul smelling, dirty liquid. The last injection did not appear to have been absorbed.

An account should be given of the pigs furnishing the blood with which the cultures were mixed.

Expt. 237, Yorkshire, wt. 29 lbs. 7-8-'09, received 10 c.c. mixed serum 264 and 1 c.c. virus Expt. 101. Remained well. 12-21-'09 killed; normal.

Expt. 323 B., wt. 36 lbs. Just received. No treatment. 12-28-'09, killed; normal.

Expt. 255, wt. 21 lbs. 7-8-09, received 12.5 c.c. mixed serum 28. 12-3-'10, killed; normal.

Expt. 193, wt. 15 lbs. 4-29-'09, received 15 c.c. mixed serum 17 and 1 c.c. bouillon culture B. cholerae suis, "No. 2 Rabbit 25" in the ear vein. 5-25-'09 injected one agar slant, 24 hours old B. cholerae suis "No. 2. Rabbit 25" in muscles. Produced abscess; opened and disinfected.

6-22-'09 injected 1 c.c. virus 99 in muscles;

7-16-'09 injected 5 c.c. virus 107 in muscles;

1-11-'10 killed; normal.

Our object was to make an artificial virus blood containing a maximum of B. cholerae suis but none of the filterable virus. There is no reason to believe that any of the blood used by us contained the filterable virus.

Virus pig 139 is to be regarded as a pig hyper-immunized to B. cholerae suis. We should expect to find in the blood serum of this pig anti-bodies responsive to the specific antigen, B. cholerae suis but none for the filterable virus (unless there is an intimate biological relation between the two).

Table I gives the results of a few agglutination tests with the blood of this pig. An increase in the agglutinative power from 1-125 to 1-25000 is noted.

We know of no test capable of disclosing to us anti-bodies for the filterable virus except the biological test, using pigs. We accordingly tested the serum, drawn from virus pig 139 against hog cholera virus by injecting small pigs. The virus used was not filtered and may have contained both the filterable virus and B. cholerae suis.

Table II shows that the serum from virus pig 139 possessed considerable potency against hog cholera virus. Small pigs were not protected against 1 c.c. of virus by 10 c.c. or 15 c.c. of this serum but protection

TABLE I

Agglutination tests with blood from virus pig 139 treated with normal pig blood mixed with cultures of B. cholerae suis.

Treatment of pig.	Date of treatment.	Date of drawing sample.	Culture used in test.
No treatment		12 7 09	Virus 122
5 cc. virus experiment 199	12 7 09		
150 cc. blood from experiment pig 137 and 50 cc. of 20 hour bouillon culture B. Cholerae Suis	12 22 09		
465 cc. blood from experiment pig 323b and 160 cc. 16 hour bouillon culture B. Cholerae Suis	12 30 09		
150 cc. blood from experiment pig 255 and 150 cc. 18 hour bouillon culture B. Cholerae Suis	1 4 10		
Bled from tail 289 cc.	1 12 10	1 12 10	Virus 139
150 cc. blood from experiment pig 193 and 150 cc. 16 hour bouillon culture B. Cholerae Suis	1 13 10		
Died	1 13 10		

Dilution of serum (A = Agglutination, S = Sediment):

Treatment of pig.	50. A	50. S	100. A	100. S	125. A	125. S	250. A	250. S	500. A	500. S	800. A	800. S	1,000. A	1,000. S
No treatment	+						0	0	0	0				
5 cc. virus experiment 199														
150 cc. blood from experiment pig 137 ...														
465 cc. blood from experiment pig 323b ...														
150 cc. blood from experiment pig 255 ...														
Bled from tail 289 cc.									+	+	+	+	+	+
150 cc. blood from experiment pig 193 ...														
Died														

Treatment of pig.	2,000. A	2,000. S	4,000. A	4,000. S	5,000. A	5,000. S	10,000. A	10,000. S	12,500. A	12,500. S	25,000. A	25,000. S	50,000. A	50,000. S
No treatment														
5 cc. virus experiment 199														
150 cc. blood from experiment pig 137 ...														
465 cc. blood from experiment pig 323b ...														
150 cc. blood from experiment pig 255 ...														
Bled from tail 289 cc.	+	+	–	–										
150 cc. blood from experiment pig 193 ...														
Died					–	–	–	–	–	–	–	–	0	0

TABLE II

Testing serum from virus pig 139 against virus 122.

No. of pig.	Weight.	Date treated.	Amount of serum.	Amount of virus	Date died.	Days lived.	Remarks.
Experiment 365	15 lbs.	1 21 10	10 cc.	1 cc.	1 30 10	9 days	Generalized hemorrhagic lesions of hog cholera.
Experiment 366	16 lbs.	1 21 10	15 cc.	1 cc.	1 30 10	9 days.	Slight hemorrhagic lesions of hog cholera.
Experiment 367	20 lbs.	1 21 10	20 cc.	1 cc.	......	......	Remained well.
Experiment 368	25 lbs.	1 21 10	25 cc.	1 cc.	......	......	Remained well.
Virus 149	87 lbs.	1 21 10	....	5 cc.	*2 1 10	11 days.	Extensive hemorrhagic lesions of hog cholera.

*Pig killed while showing symptoms of hog cholera.

was offered by 20 c.c. and 25 c.c. That the virus used was virulent is shown by the death of Expt. pigs 365 and 366 and by the development of hog cholera in virus pig 149.

Should we conclude that anti-bodies against the filterable virus are produced as a result of using B. cholerae suis for the antigen? The Experiment points strongly to the possibility of using cultures of B. cholerae suis in normal pig blood for hyper-immunizing serum hogs.

Possibly the results of this experiment depended partly upon the blood used in connection with the cultures.

We, accordingly, attempted to hyperimmunize an immune pig with cultures of B. cholerae suis only. For this experiment, we used the same strain employed in the preceding experiment and Expt. pig 315.

Expt. pig 315. Chester white, weight 37 lbs.

On 11-30-'09 injected 25 c.c. mixed serum 41 and 1 c.c. virus 128. Pig remained well.

On 2-1-'10 injected 10 c.c. virus 149. Pig remained well for over three weeks, showing immunity to hog cholera virus.

On 2-24-'10 injected 10 c.c. of a 24 hour bouillon culture of B. cholerae suis into axillary region. Estimates from agar plates indicated about 500 million bacteria per c.c.

No local lesion could be detected on 3-3-'10 when 45 c.c. of a 24 hour bouillon culture of B. cholerae suis was injected into the axillary region.

On 3-11-'10 injected into axillary region 45 c.c of a 24 hour bouillon culture of B. cholerae suis. Platings on agar indicated the presence of from one-half to one billion bacteria per c.c. No visible or palpable lesions locally.

On 3-19-'10 injected 40 c.c. of a 24 hour bouillon culture of B. cholerae suis into the axillary region. Platings indicated the presence of about one billion bacteria per c.c. No very distinct swelling locally but considerable soreness. Bled 180 c.c. from tail, defibrinated and preserved in 5% carbolic acid, 10 c.c. to 90 c.c. of blood.

On 4-2-'10 bled from tail 289 c.c., defibrinated and preserved in carbolic acid.

On 4-27-'10 bled from tail 400 c.c., defibrinated and preserved in carbolic acid. Mixed bleedings of 3-19-'10, 4-2-'10, and 4-27-'10. Labeled, "Mixed Serum Expt. 315."

On 5-20-'10 drew sample from tail for agglutination test.

On 6-8-'10 drew sample from tail for agglutination test. Pig weighed 143 lbs. on 6-24-10.

Killed, 7-1-'10. In good condition.

Autopsy: In each axillary region were extensive abscesses, well defined and encapsulated with no indication of infiltration into the surrounding tissues. Contents of abscesses, a dirty yellowish-brown, thick *liquid with foul odor. Inoculations gave B. cholerae suis in pure culture. Other organs, normal. Pieces of spleen, kidney, liver and heart produced no growth in bouillon.*

Table III shows the results of agglutination tests with samples of serum from Expt. pig 315 drawn at various times. There is manifestly a great stimulus to the production of agglutinins. The mixed serum used in the biological test (vide Table IV) give a reaction at 1-500,000. We have previously found that all samples of Dorset-Niles serum

TABLE III.

Agglutination tests with blood from experiment pig 315 treated with cultures of B. cholerae suis and culture "virus 136".

Dilution of serum.			25.		50.		100.		125.		250.		500.		1,000.	
Treatment of pig.	Date of treatment.	Date of drawing sample.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.
No treatment		11-30-09	0	0	0	0	0	0	0	0	0	0				
25 cc. Mixed serum 41; 1 cc. virus 128	11-30-09	1-21-09			+	+	+	+								
10 cc. virus 149	2-1-10															
10 cc. 24 hour bouillon culture B. cholerae suis "Virus 136"	2-24-10	3-3-10			+	+	+	+	+	+	−	−				
45 cc. 24 hour bouillon culture B. cholerae suis "Virus 136"	3-3-10	3-12-10											+	+	+	+
45 cc. 24 hour bouillon culture B. cholerae suis "Virus 136"	3-11-10															
Bled 180 cc. from tail	3-19-10	3-19-10											+	+	+	+
40 cc. 24 hour bouillon culture B. cholerae suis "Virus 136"	4-2-10															
Bled 289 cc. from tail	4-2-10	4-2-10											+	+	+	+
Bled 300 cc. from tail	4-14-10	4-14-10														
Bled 400 cc. from tail	4-27-10	4-27-10														
Bled 10 cc. from tail	5-20-10	5-20-10														
Bled 10 cc. from tail	6-8-10	6-8-10														
Mixed sample																
Killed	7-1-10	7-1-10														

Dilution of serum.	2,000.		4,000.		5,000.		10,000.		12,500.		25,000.		50,000.		100,000.		125,000.		250,000.		500,000.	
Treatment of pig.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.
No treatment																						
25 cc. Mixed serum 41; 1 cc. virus 128																						
10 cc. virus 149																						
10 cc. 24 hour bouillon culture B. cholerae suis "Virus 136"																						
45 cc. 24 hour bouillon culture B. cholerae suis "Virus 136"	+	+																				
45 cc. 24 hour bouillon culture B. cholerae suis "Virus 136"																						
Bled 180 cc. from tail	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							
40 cc. 24 hour bouillon culture B. cholerae suis "Virus 136"																						
Bled 289 cc. from tail	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	−	−		−
Bled 300 cc. from tail															−	−	−	−	−	−	−	−
Bled 400 cc. from tail																	−		0	0	0	0
Bled 10 cc. from tail					+	+																
Bled 10 cc. from tail					+	+	+	+					0	0								
Mixed sample													+	−	−	−	−	−	−	−	−	−
Killed					+	−	+	−	+	−	−	−		−								

TABLE IV.

Testing mixed serum experiment 315 against virus 170.

No. of pig	Weight.	Date treated.	Amount of serum	Amount of virus	Date died.	Days lived	Remarks
Experiment 395	15 lbs.	4 27 10	10 cc.	1 cc.	5 5 10	8 days	Generalized hemorrhagic lesions of hog cholera.
Experiment 396	15 lbs.	4 27 10	15 cc.	1 cc.	5 5 10	8 days	Generalized hemorrhagic lesions of hog cholera.
Experiment 397	15 lbs.	4 27 10	20 cc.	1 cc.	5 3 10	6 days	Generalized hemorrhagic lesions of hog cholera.
Experiment 398*	15 lbs.	4 27 10		1 cc.	5 3 10	6 days	Generalized hemorrhagic lesions of hog cholera.

*Killed in dying condition.

that react at a dilution of 1-50,000 are potent. Here we have a serum produced in a peculiar manner with a high agglutinative power, but with little or no potency.

Table IV give the results of the test for potency of the serum from Expt. pig 315 on small pigs. The serum was tested against a very virulent unfiltered hog cholera virus. We found no indication of any protective power in this serum. Perhaps larger doses might have revealed the presence of protective substances.

We next attempted to eliminate B. cholerae suis from the process of hyperimmunization. Our object was to hyperimmunize a pig with virus blood free from any cultivable organisms. It proved to be a herculean task on account of the slow filtration and frequent presence of cloudiness in our filtrates and especially on account of our mistake in selecting a large pig for the experiment. We planned to dilute with equal parts of sterile .85 per cent NaCl solution the clear serum from defibrinated hog cholera virus blood and filter through both Berkefeld and Chamberland filters. We were then to inject 20 c.c. of this filtrate for each pound of live weight of the pig. It was soon discovered that our facilities for filtering the virus blood could not keep pace with the increase in weight of the pig.

Expt. pig 387, Yorkshire, weight 16 lbs.

Feb. 7, 1910, injected 20 c.c. mixed serum 53 and 1 c.c. virus 125.

June 24, weighed 81 lbs.

August 27, injected 250 c.c. virus Expt. 431 and 250 c.c. virus 205. Both lots were clear blood serum diluted with equal parts of sterile .85 per cent NaCl solution and filtered through Berkefeld, then through Chamberland filters.

Two Berkefeld filters were used for virus Expt. 434 and one new Chamberland candle at air pressure of about 3 Kg. per square centimeter. Incubated 3 days, no growth. Virus 205 put through 3 Berkefeld filters and one Chamberland at 2 to 3 Kg. pressure. Incubated 4 days, no growth.

Sept. 1, injected 75 c.c. virus 208, 125 c.c. virus 209, and 50 c.c. virus 211 diluted and filtered as above. Virus 208 put through one Berkefeld and two Chamberland filters at 3 to 4 Kg. pressure. Incubated 2 days, no growth. Virus 209 put through 3 Berkefeld filters and one Chamberland very slowly. Incubated 5 days, no growth.

Sept. 7, injected 25 c.c. virus 208 and 225 c.c. virus 210. Diluted and filtered as above. Virus 210 put through one Berkefeld and one Chamberland filter at 3 to 4 Kg. pressure. Incubated 7 days, no growth.

Sept. 14, injected 75 c.c. virus 211 and 125 c.c. virus 207. Prepared as above. Virus 211 filtered through 2 Berkefeld and one Chamberland filter very slowly. Virus 207 put through one Berkefeld and one Chamberland filter. Incubated 6 days, no growth.

Sept. 20, injected 100 c.c. virus 207, a portion of above Berkefeld filtrate passed through one Chamberland filter 9-13-'10. Incubated 7 days, no growth. The pig weighed 138 lbs. on this date.

Sept. 28, injected 400 c.c. virus 223. Prepared as above. Filtered through one Berkefeld and one Chamberland. Incubated 6 days, no growth.

Oct. 5, injected 500 c.c. of a mixture of virus Expt. 435 to 459. Prepared as above. Filtered through 3 Berkefeld filters and 5 Chamber-

land candles. One portion clouded, others clear after 2 days incubation.

Oct. 15, injected 70 c.c. virus Expt. 453 to 459. This was one of the portions described above.

Oct. 26, injected 50 c.c. virus 224 and 200 c.c. virus 227. Prepared as above: virus 224 filtered through one Chamberland filter only. Virus 227 filtered through four Chamberland filters. Both samples incubated and remained clear.

Nov. 2, bled *from* tail enough to make 663 c.c. after defibrinating and adding .5 per cent trikresol. Injected 75 c.c. virus 224 and 225 c.c. virus 229. Prepared as above. Virus 224 was a portion of that described above. Virus 229 filtered through one Berkefeld and 3 Chamberland filters. Used only first filtrate.

Nov. 9, killed. Dressed 122 lbs. Secured 1939 c.c. defibrinated blood preserved in .5 per cent trikresol. All the injections had been made *in the axillary region.* There was never any swelling or soreness. At autopsy, only a yellowish-brown discoloration of the tissues at point of inoculation was discovered.

The records show that a total of 2820 c.c. of diluted virus was used to hyperimmunize Expt. 387. The pig weighed about 160 lbs. when killed. There was, therefore, used only about 17.5 cc. of diluted virus *instead of 20 c.c. per pound live weight.*

Samples of both bleedings were tested for agglutinative power against B. cholerae suis. Table V shows results of agglutination tests, with the *tail bleeding and last bleeding* of Expt. pig 387.

TABLE V.

Agglutination tests with serum from experiment 387 and B. cholerae suis.

Dilution.	Tail bleeding.		Last bleeding.	
	Agglutination.	Sediment.	Agglutination.	Sediment.
1-50	+	−	+	+
1-100	−	+	+	+
1-125	+	+	+	+
1-250	+	+	+	+
1-500	+	+	+	+
1-800	+	+	+	+
1-1600	−	−	−	−
1-2000	−	−	−	−
1-4000	−	−	−	−
1-8000	−	−	−	−

The agglutinative power of this serum is as great as that of nearly two-thirds of the samples of Dorset-Niles serum prepared in the usual way. *Unfortunately, we failed to test the agglutinative power of the blood of this pig before making the filtered virus injections.* We have previously found that pigs treated by the serum-simultaneous method

as Expt. 387 was treated do not furnish a serum agglutinating at a dilution higher than 1-500. We think we are justified in concluding that the agglutinative power of the blood of Expt. 387 increased from 1-500 to 1-8000 during the injections of filtered virus which contained no organisms capable of producing cloudiness in the diluted filtrate.

Another evidence of the absence of B. cholerae suis in the filtrate lies in the fact that no abscesses were produced as a result of injecting extra large volumes of diluted virus. It is a common experience to find that the injections of unfiltered virus blood produce the so-called B. cholerae suis abscesses or caverns filled with a thick, dirty fluid usually walled off by connective tissue. We must admit our ignorance of the cause of the increase in agglutinative power of the serum from Expt. 387.

The potency of the serum from Expt. 387 was tested on small pigs against both the filtered and unfiltered hog cholera virus. The two bleedings were mixed and termed "Mixed Serum Expt. 387." Table VI shows the tests made with mixed serum Expt. 387 against virus Expt. 536. Table VII shows the tests made against virus Expt. 536 diluted with equal parts of .85% NaCl solution and filtered through a Chamberland filter.

It is seen from a study of these two tables that the serum, drawn from a pig hyperimmunized with filtered virus only, protects against either the filtered or unfiltered virus. However, the protective power against the filtered virus is much greater than against the unfiltered virus. Only the pig receiving 5 c.c. of serum dies of cholera when treated with the unfiltered virus, whereas the pig receiving 20 c.c. of serum together with the unfiltered virus dies of cholera. These experiments tend to show that in filtering hog cholera virus through a Chamberland filter, something is removed that has considerable to do with producing hog cholera. Perhaps the filter has an effect of only weakening the virus by permitting the serum to pass but retaining a proportionately large number of the ultra-microscopic organisms, rather than by keeping back a virulent pathogens of an entirely different species such as B. cholerae suis. While it is hardly worth while to speculate, it may be of interest to note that the suggestion has been made that the filterable virus, incapable itself of growing in vitro, is only a peculiar form of a microscopic organism which is capable of growing in vitro. In this case, of course, filtration would weaken the virus by removing a portion of its active principle.

The experiments recorded in Tables VIII and IX furnish additional evidence for the belief that it is easier to protect against the filtered virus than against the unfiltered hog cholera blood. Table VIII shows that 10 c.c. and 15 c.c. of Mixed Serum 35 furnish complete and enduring protection to small pigs against hog cholera when injected simultaneously with 2 c.c. of the filtrate of virus 136 diluted with equal parts of .85 per cent NaCl solution and passed through a Chamberland filter. The filtrate furnished no growth when incubated. Table IX shows that neither 10 c.c. nor 15 c.c. of Mixed Serum 35 protects against 2 c.c. of virus 136 diluted with equal parts of .85 per cent NaCl solution and not filtered. Virulent cultures of B. cholerae suis were found in pure culture in the blood and organs of virus pig 136.

TABLE VI.

Testing mixed serum experiment 387 against virus experiment 536 diluted and filtered.

No. of pig.	Weight.	Date treated.	Amount of serum.	Amount of diluted virus.	Date died.	Days lived.	Remarks.
Experiment 545	11 lbs.	12-9-10	5 cc.	2 cc.	1-8-11	30 days	Hemorrhagic lesions and ulcers.
Experiment 546	17 lbs.	12-9-10	10 cc.	2 cc.			Remained well.
Experiment 547	16 lbs.	12-9-10	15 cc.	2 cc.	2-8-11	61 days	No cholera lesions. Emaciated, ascarides and lice.
Experiment 548	18 lbs.	12-9-10	20 cc.	2 cc.	3-28-11	109 days	No cholera lesions. Emaciated, ascarides and lice.
Experiment 549	30 lbs.	12-9-10	25 cc.	2 cc.			Remained well.
Experiment 550	14 lbs.	12-9-10		2 cc.	12-18-10	9 days	Acute hemorrhagic cholera.
Experiment 551	13 lbs.	12-9-10		2 cc.	12-16-10	8 days	Acute hemorrhagic cholera.

TABLE VII.

Testing mixed serum experiment 387 against virus experiment 533 neither diluted nor filtered.

No. of pig.	Weight.	Date treated.	Amount of serum.	Amount of virus.	Date died.	Days lived.	Remarks.
Experiment 552	9 lbs.	12-9-10	5 cc.	1 cc.	12-12-10	3 days	Liver gorged with blood.
Experiment 553	12 lbs.	12-9-10	10 cc.	1 cc.	12-14-10	5 days	Pleuro-pneumonia and hemorrhages.
Experiment 554	18 lbs.	12-9-10	15 cc.	1 cc.			Remained well.
Experiment 555	10 lbs.	12-9-10	20 cc.	1 cc.	1-6-11	28 days	Hemorrhagic lesions and ulcers.
Experiment 556	20 lbs.	12-9-10	25 cc.	1 cc.			Remained well.
Experiment 557	17 lbs.	12-9-10		1 cc.	12-16-10	7 days	Hemorrhagic lesions.
Experiment 558	12 lbs.	12-9-10		1 cc.	12-15-10	6 days	Hemorrhagic lesions.

TABLE VIII.

Testing mixed serum 35 against virus 1:36 diluted and filtered.

No. of pig.	Weight.	Date treated.	Amount of serum.	Amount of diluted virus.	Date died.	Days lived.	Remarks.
Experiment 317	50 lbs.	12-15-09	10 cc.	2 cc.			1-4-10 injected 2 cc. virus 142; 4-26-10 injected 100 cc. virus 172; made Ser. Hog 162.
Experiment 318	50.5 lbs.	12-15-09	15 cc.	2 cc.			1-4-10 injected 2 cc. virus 142; 4-23-10 injected 100 cc. virus 174; made Ser. Hog 158.
Experiment 319	48.5 lbs.	12-15-09		2 cc.	1-19-10	35 days.	1-4-10 injected 2 cc. virus 142. Hemorrhages and ulcers.

TABLE IX.

Testing mixed serum 35 against virus 1:36 diluted but not filtered.

No. of pig.	Weight.	Date treated.	Amount of serum.	Amount of diluted virus.	Date died.	Days lived.	Remarks.
Experiment 320	44 lbs.	12-15-09	10 cc.	2 cc.	1-15-10	31 days	Hemorrhagic lesions and ulcers.
Experiment 321	41.5 lbs.	12-15-09	15 cc.	2 cc.	12-25-09	10 days	Hemorrhagic lesions extensive.
Experiment 322	53 lbs.	12-15-09		2 cc.	12-26-09	9 days	Hemorrhagic lesions extensive.

TABLE X.

Testing mixed serum 65 against 1 cc. virus experiment 442.

No. of pig.	Approximate weight.	Date treated.	Amount of serum.	Amount of virus.	Date died.	Days lived.	Remarks.
Experiment 460	20 lbs.	10 4 10	5 cc.	1 cc.	12 6 10	63 days	Hemorrhagic lesions.
Experiment 461	20 lbs.	10 4 10	10 cc.	1 cc.	......	....	Remained well.
Experiment 462	20 lbs.	10 4 10	*3 cc.	*1 cc.	10 26 10	22 days.	Hemorrhagic lesions.
Experiment 463	20 lbs.	10 4 10	*4 cc.	*1 cc.	11 7 10	34 days	Hemorrhagic lesions.
Experiment 464	20 lbs.	10 4 10	*5 cc.	*1 cc.	11 18 10	45 days	Hemorrhagic lesions and ulcers.
Experiment 465	20 lbs.	10 4 10	....	1 cc.	10 41 10	10 days	Hemorrhagic lesions.

*Serum and virus mixed in test tube and incubated at 37° C. for 3 hours.

TABLE XI.

Testing mixed serum 78 against virus experiment 475.

No. of pig.	Approximate weight.	Date treated.	Amount of serum.	Amount of virus.	Date died.	Days lived.	Remarks.
Experiment 523	30 lbs.	10 29 10	5 cc.	1 cc.	11 10 10	12 days	Hemorrhagic lesions.
Experiment 524	30 lbs.	10 29 10	10 cc.	1 cc.	11 10 10	12 days	Hemorrhagic lesions.
Experiment 525	30 lbs.	10 29 10	15 cc.	1 cc.	....	....	Remained well.
Experiment 526	30 lbs.	10 29 10	*3 cc.	*1 cc.	11 10 10	12 days.	Hemorrhagic lesions.
Experiment 527	30 lbs.	10 29 10	*4 cc.	*1 cc.	11 10 10	12 days	Hemorrhagic lesions.
Experiment 528	30 lbs.	10 29 10	*5 cc.	*1 cc.	11 14 10	18 days	Hemorrhagic lesions.

*Serum and virus mixed in test tube and incubated two and one-half hours.

We believe that the title of this paper will permit us to include a few experiments which aimed to determine the effect of the Dorset-Niles serum on hog cholera virus in vitro. We have absolutely no definite knowledge as to the action in vivo of the Dorset-Niles serum. Whether it is antimicrobial, antitoxic, or a promoter of phagocytosis by stimulating the production of opsonins we do not know. It is not easy to determine any of these points. If the serum is antimicrobial, it appears to us that its action on the living cause of hog cholera should take place in a mixture of the two in vitro. If its action is antitoxic, it should at least neutralize any antitoxin produced in vitro. The determination of phagocytosis of an ultravisible organism will require a technic specially devised for the purpose, and probably dissimilar to any with which we are now familiar.

We have worked upon the hypothesis that if a given amount of serum is required to protect a pig against a given amount of virus when they are injected into the pig simultaneously then a less amount of serum will be required to protect against the same amount of virus if the two are mixed in vitro and allowed to remain several hours before injecting into the system of the pig. If a less amount is required then there is evidence of antimicrobial or antitoxic action, probably the former, but if an equal amount of serum is required, then there is no evidence of such action.

Table X shows that 10 c.c. of Mixed Serum 65 protects against 1 c.c. of virus Expt. 442, but that 5 c.c. does not, either when used in the simultaneous method or after mixing with virus and incubating three hours. As a matter of fact, death occurs eighteen days sooner when the mixture is used than when the serum and virus are used separately.

Table XI is not very instructive in this connection, since the disparity is great between the amount of serum that is necessary to protect in the simultaneous method and the maximum amount that was used in the in vitro tests. It is seen that 15 c.c. is necessary to protect by the ordinary method, while we used only 5 c.c. in the mixture after incubating two and one-half hours. We have not proved that there is no in vitro action, since our work is too limited in character, but our results indicate that it is doubtful whether this line of experimentation will be fruitful of results.

SUMMARY.

1. Three pigs were hyperimmunized by injections of (a) B. cholerae suis mixed with pig blood free of the filterable virus, (b) pure cultures of B. cholerae suis, and (c) the filtered virus free of B. cholerae suis respectively.

2. The serum from the pig hyperimmunized with B. cholerae suis mixed with blood protected a 20 lb. pig in 20 c.c. dose and a 25 lb. pig in 25 c.c. dose. This serum also agglutinated B. cholerae suis at a dilution of 1-12,500.

3. The serum from the pig hyperimmunized with pure cultures of B. cholerae suis furnished no protection to a 15 lb. pig in doses of 20 c.c. This serum agglutinated B. cholerae suis when diluted 1-500,000.

4. The pig hyperimmunized with the filtered virus free of B. cholerae suis furnished a serum capable of protecting a 17 lb. pig in 10 c.c. dose

against the filtered virus and an 18 lb. pig in 15 c.c. dose against the unfiltered virus. This serum also agglutinated B. cholerae suis when diluted 1-8,000.

5. It was found that the Dorset-Niles serum is more potent against the filtered virus than against the unfiltered virus.

6. The mixing of the Dorset-Niles serum and hog cholera virus in vitro and incubating 2½ to 3 hours does not seem to lessen the virulence of the virus. There is no indication that mixing the serum and virus in vitro has any advantage over the serum-simultaneous method of injecting these agents for immunizing pigs against hog cholera.